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TITLE: DOES LACTATION MITIGATE TRIPLE NEGATIVE/BASAL BREAST CANCER PROGRESSION?

PRINCIPAL INVESTIGATOR: Tanya Russell, Ph.D.

CONTRACTING ORGANIZATION: University of Colorado Anschutz Medical Campus
                           Aurora, CO 80045

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E-Mail: tanya.russell@ucdenver.edu	

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14. ABSTRACT

The prevalence and mortality rate of "triple negative/basal breast cancer" is increased young African American women compared to young Caucasian women. We hypothesize that pregnancy and involution without lactation promotes triple negative/basal breast cancer. Aims: 1) Determine if pregnancy and involution, in the absence of lactation, confers tumor promotion. 2) Determine if lactation mitigates promotional effects of pregnancy/involution on tumor progression. Results: Compared to nulliparous controls, tumors in the pregnant group appear to be less proliferative and tumor burden is not increased. In contrast, tumors in the involution group were much more proliferative and tumor burden was increased with respect to nulliparous controls. The protective myoepithelial cell layer may be preferentially compromised by tumors formed in post-partum involution mammary glands. These data may reflect key differences in the influence of these reproductive states on tumor progression. Impact: Our initial studies help to address the question of why breast cancer (especially in African American women), when diagnosed within close proximity to a recent pregnancy, is more aggressive and more likely to spread.

15. SUBJECT TERMS
Triple negative/basal breast cancer, pregnancy-asssociated breast cancer, young African American women, pregnancy, lactation, involution

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INTRODUCTION

Young African American women have increased risk of the poor prognostic triple negative/basal breast cancer subtype and higher mortality compared to young Caucasian women with breast cancer [1-3]. Further, in all young women, breast cancers associated with a recent pregnancy (pregnancy-associated breast cancer, or PABC) have poor prognosis because death from metastases is more common [4-9]. Based on epidemiologic data demonstrating increased parity in African American women compared to Caucasian populations [10, 11], we posit that the gestational hormones of pregnancy combined with the pro-inflammatory, wound-healing environment of involution provide a two-hit proliferative/remodeling microenvironment that promotes metastasis in triple negative/basal breast cancer. Recent epidemiologic data suggest that increasing length of time between pregnancy and involution via lactation could reduce promotion of aggressive breast cancers in African American women [10, 12]; however, experimental data supporting this hypothesis is lacking. We hypothesize that pregnancy and involution without lactation provide a two hit proliferative/remodeling microenvironment that promotes breast cancer. **Specifically, we predict that the triple negative/basal breast cancer subtype is promoted by pregnant and involuting mammary microenvironments but this promotion is mitigated if lactation ensues.**

To test our hypothesis, we will utilize mouse intraductal xenograft models [13, 14] developed in our laboratory to investigate whether the tumor promotional microenvironments of pregnancy and involution enhance progression of triple negative/basal human breast cancer cells and if lactation mitigates the progression of these cells. The results of these studies are anticipated to provide mechanistic insight into the poor prognosis of pregnancy associated breast cancer; delineate the promotional effects of pregnancy from those of post-partum involution, identify potential crosstalk between these physiologic windows of increased breast cancer risk, and corroborate epidemiologic data supporting a protective role of lactation on aggressive breast cancer progression. Young African American women, and particularly recently pregnant African American women, are at a greater risk of dying from triple negative/basal breast cancer than Caucasian women. Hopefully, this research will create a bridge between basic science and health disparity research by establishing the lactogenic microenvironment as a paradigm for future preventative strategies, therapeutic intervention, and/or treatment in young African American women. Not only could the breast cancer health disparity that exists in this population be reduced, but aggressive breast cancers in all women could potentially be mitigated.

BODY

STATEMENT OF WORK AND ACCOMPLISHMENTS

Specific Aim 1 – Determine if pregnancy and/or involution, in the absence of lactation, confer tumor promotion of triple negative/basal breast cancer.

Task 1 – Develop intraductal xenograft mouse model of triple negative/basal breast cancer during pregnancy. Months 1-12

1a. Randomize 48 SCID mice, 5-weeks of age, into 2 groups (nulliparous control and day 19 of gestation) with 12 mice/group for each triple negative/basal cell line (MCF10ADCIS.com and MDA-MB-157). Task completed for MCF10ADCIS.com cell line.

1b. Intraductally inject either MCF10ADCIS.com or MDA-MB-157 into left and right 3rd and 4th mammary glands of all mice at age 5 weeks. Task completed for MCF10ADCIS.com cell line.

1c. Breed mice except the nulliparous controls at 10 weeks of age. Task completed MCF10ADCIS.com cell line.

1d. Euthanize pregnant mice on day 19 of gestation along with age-matched nulliparous controls. Tumor "age" at this stage is 8 weeks. <mark>Task completed for MCF10ADCIS.com cell line.</mark>

1e. Harvest the left and right 3rd and 4th mammary glands 8 weeks post-injection and fix in formalin for histology. <mark>Task completed for MCF10ADCIS.com cell line.</mark>

Major Updates for Tasks 1a – 1e:

1. In our intraductal preclincal model of human breast cancer, MCF10ADCIS.com cells undergo evolution from stages of DCIS development (hyperplasia, solid, cribriform, papillary, and comedo) to invasive lesions in both the nulliparous and pregnant host environments as assessed by hemotoxylin and eosin histology (Figure 1).

2. Tumor burden was not increased in the pregnant group compared to their respective nulliparous controls (Figure 2A). Ki67 proliferative index (Figure 2B) is increased in tumors from the nulliparous group compared to normal cells ($^{\dagger}p = 1.956E-06$). Interestingly, there was no difference in Ki67 proliferative index between tumors and normal cells in the pregnant group ($p = 0.1256$). There was a slight but significant decrease in Ki67 proliferative index in tumors from the pregnant group compared to tumors from their respective nulliparous controls ($^{\dagger\dagger}p = 0.0441$). As expected, Ki67 proliferative index was much higher in normal cells from the pregnant group compared to nulliparous controls ($^{\dagger\dagger\dagger}p = 1.407E-05$).

3. Although MCF10ADCIS.com cells were initially characterized as being triple negative [15], some MCF10ADCIS.com tumors re-express estrogen receptor (ER) in vivo (Figure 3A). There was a slightly lower but significant (*$p = 0.0697$) expression of ER in the pregnant group compared to their respective nulliparous controls (Figure 3B). Thus, tumors in the pregnant group appear to be less proliferative and have slightly lower ER expression than tumors in their respective nulliparous controls, suggesting that the hormonal milieu of pregnancy may have an effect on certain mechanisms of tumor progression.

4. In the pregnant host environment myoepithelial cells surrounding DCIS lesions have higher expression of smooth muscle actin and calponin compared to their respective nulliparous controls, suggesting a more differentiated phenotype. Myoepithelial cell expression of p63, a putative tumor suppressor, is lost early during DCIS progression independent of group (Figure 4 and Table 1).

Proposed Work for Tasks 1a – 1e, Year Two:

1. Submit manuscript characterizing the effect of pregnancy and involution on early stages of tumor progression (currently in progress).

2. Submit manuscript describing the technical aspects of the intraductal injection method (currently in progress).

3. Continue characterizing changes in the expression of myoepithelial cell markers with respect to tumor progression in the pregnant and nulliparous host environments.

4. Develop intraductal xenograft mouse model of triple negative/basal breast cancer during pregnancy using the MDA-MB-157 cell line.

1f. Harvest the liver, lung, kidney and brain tissues for determination of metastatic lesions by both IHC and PCR based techniques. <mark>Task completed for MCF10ADCIS.com cell line.</mark>

Major Updates for Task 1f:
1. We currently have paraffin embedded tissue from liver, lung, kidney and brain from both nulliparous and pregnant groups and lung serial sections cut from nulliparous and pregnant groups.

2. We have also collected frozen tissue from liver, lung, kidney and brain from both nulliparous and pregnant groups to assess metastases by QRT-PCR analysis.

Proposed Work for Task 1f, Year Two:
1. Cut serial sections throughout entire tissues from liver, kidney and brain from both nulliparous and pregnant groups to analyze presence of metastatic lesions.

2. Perform QRT-PCR analysis to assess metastases.

Task 2 – Develop our intraductal xenograft model of pregnancy associated breast cancer to determine whether the involution microenvironment alone promotes triple negative/basal metastasis. Months 1-12

1a. Randomize 48 SCID mice, 5-weeks of age, into 2 groups (nulliparous control and day 19 of gestation) with 12 mice/group for each triple negative/basal cell line (MCF10ADCIS.com and MDA-MB-157). Task completed for MCF10ADCIS.com cell line.

1b. Breed mice at 5 weeks of age except the nulliparous controls. After parturition, normalize pups to 8, and wean at 9 days of parturition to initiate involution. Task completed for MCF10ADCIS.com cell line.

1c. Intraductally inject either MCF10ADCIS.com or MDA-MB-157 into left and right 3rd and 4th mammary glands of mice 2 days post-weaning (also inject age-matched nulliparous controls). Task completed for MCF10ADCIS.com cell line.

1d. Euthanize all mice 8 weeks post-injection. Tumor "age" at this stage is 8 weeks. Task completed for MCF10ADCIS.com cell line.

1e. Harvest the left and right 3rd and 4th mammary glands 8 weeks post-injection and fix in formalin for histology. Task completed for MCF10ADCIS.com cell line.

Major Updates for Task 2 (1a – 1e):
1. In our intraductal preclinical model of human breast cancer, MCF10ADCIS.com cells undergo evolution from stages of DCIS development (hyperplasia, solid, cribriform, papillary, and comedo) to invasive lesions in both the nulliparous and involution host environments as assessed by hemotoxylin and eosin histology (Figure 5A) and fluorescent in situ hybridization analyses (Figure 5B).

2. Tumor burden is greater in the involution group compared to their respective nulliparous controls (Figure 6A). Ki67 proliferative index (Figure 6B) is increased in tumors from both the nulliparous and involution groups compared to normal cells (*p = 5.859E-08 and 1.082E-07, respectively). There was no significant difference in Ki67 proliferative index between tumors in the nulliparous group compared to the involution group (p = 0.1737). However, there was a decrease in Ki67 proliferative index in normal cells from the involution group compared to normal cells from their respective nulliparous controls (**p = 0.021).

3. There was no difference in ER expression in tumors from the involution group compared to their respective nulliparous controls (*p = 0.3009) (Figure 7).

4. In myoepithelial cells surrounding post-partum involution group DCIS lesions, smooth muscle actin expression is high, but calponin is lost at a higher frequency, suggesting a less differentiated phenotype. Myoepithelial cell expression of p63, a putative tumor suppressor, is lost early during DCIS progression independent of group (Figure 8 and Table 2). Taken together with the pregnancy data (Figure 4 and Table 1) SMA positivity remains relatively stable, suggesting that the actin biostructure may be a later barrier broken in tumor progression. In contrast, p63 positivity appears to be lost first in all tested microenvironments and calponin positivity appears to lost preferentially in the post-partum involution environment. These data may reflect key differences in the influence of these reproductive states on tumor progression.

Proposed Work for Task 2 (1a – 1e), Year Two:
1. Submit manuscript characterizing the effect of pregnancy and involution on early stages of tumor progression (currently in progress).

2. Continue characterizing changes in the expression of myoepithelial cell markers with respect to tumor progression in the involution and nulliparous host environments.

3. Develop intraductal xenograft mouse model of triple negative/basal breast cancer during involution using the MDA-MB-157 cell line.

1f. Harvest the liver, lung, kidney and brain tissues for determination of metastatic lesions by both IHC and PCR based techniques. Task completed for MCF10ADCIS.com cell line.

Major Updates for Task 2 (1f):
1. We did not detect lung metastasis at 4 weeks post-intraductal injection in neither the involution nor nulliparous groups by QRT-PCR (Figure 9). We also did not detect lung or liver metastasis at 8 weeks post-intraductal injection by FISH analysis (Figure 10).

Proposed Work for Task 2 (1f), Year Two:
1. Determine if metastasis occurs in the intraductal injection model by increasing "tumor age" in the involution and nulliparous host environments.

Task 3 – Develop a two-hit pregnancy/involution tumor promotional intraductal xenograft model in the absence of lactation. Months 12-24

1a. Randomize 48 SCID mice, 8-weeks of age, into 2 groups (nulliparous control and day 19 of gestation) with 12 mice/group for each triple negative/basal cell line (MCF10ADCIS.com and MDA-MB-157). Task completed for MCF10ADCIS.com cell line with 18 mice.

1b. Breed mice at 8 weeks of age except the nulliparous controls. Task completed for MCF10ADCIS.com cell line with 18 mice.

1c. Intraductally inject either MCF10ADCIS.com or MDA-MB-157 into left and right 3rd and 4th mammary glands of mice at day 1 of pregnancy (also inject age-matched nulliparous controls). Task completed for MCF10ADCIS.com cell line with 18 mice.

1d. Remove pups at parturition and allow mammary glands to involute 1 week and fully regress 4 weeks. Task completed for MCF10ADCIS.com cell line with 18 mice.

1e. Euthanize all mice at 4 weeks full-regression. Tumor "age" at this stage is 8 weeks. <mark>Task completed for MCF10ADCIS.com cell line with 18 mice.</mark>

1f. Harvest the left and right 3rd and 4th mammary glands and fix in formalin for histology. <mark>Task completed for MCF10ADCIS.com cell line with 18 mice.</mark>

1g. Harvest the liver, lung, kidney and brain tissues for determination of metastatic lesions by both IHC and PCR based techniques. <mark>Task completed for MCF10ADCIS.com cell line with 18 mice.</mark>

> **Major Updates for Task 3:**
> 1. We have started one initial study with 18 mice and harvested mammary glands and other tissue.
>
> **Proposed Work for Task 3, Year Two:**
> 1. Complete intraductal injections with remaining proposed number of mice during pregnancy and involution in the absence of lactation.
>
> 2. Evaluate tumor latency, growth, burden and multifocal nature of tumor.
>
> 3. Characterize changes in the expression of myoepithelial cell markers with respect to tumor progression in absence of lactation.
>
> 4. Characterize tumor desmosplasia, proliferation, angiogenesis and lipogenesis by IHC.
>
> 5. Analyze evidence of metastasis by QRT-PCR, IHC and FISH.

Task 4 – Manuscript Preparation. Months 18-24

> **Proposed Work for Task 4, Year Two:**
> 1. Submit manuscript characterizing the effect of pregnancy and involution in the absence of lactation on tumor progression.

Specific Aim 2 – Determine if lactation mitigates promotional effects of pregnancy/involution on triple negative/basal-like breast tumor progression.

Task 1 – Develop an intraductal xenograft mouse model of triple negative/basal-like breast cancer to determine if length of lactation mitigates tumor promotion. Months 24-36

1a. Randomize 60 SCID mice, 8-weeks of age, into 5 groups of increasing lengths of lactation with 12 mice/group for each triple negative/basal cell line (MCF10ADCIS.com and MDA-MB-157).

Group #	Tumor cell Injection	Gestation (weeks)	Lactation (weeks)	Post-partum involution (weeks)	Full Regression (weeks)	End of study tumor "age" (weeks)
1	P1	3	0	1	4	8
2	P1	3	0.28 (2 days)	1	4	8
3	P1	3	1.5	1	2.5	8
4	P1	3	3	1	1	8
5	P1	3	4	1	0	8

1b. Breed mice at 8 weeks of age except the nulliparous controls.

1c. Intraductally inject either MCF10ADCIS.com or MDA-MB-157 into left and right 3rd and 4th mammary glands of mice at day 1 of pregnancy (also inject age-matched nulliparous controls.

1d. Euthanize animals at various stages of full gland regression for tumor "age" to be 8 weeks throughout entire study.

1f. Harvest the left and right 3rd and 4th mammary glands and fix in formalin for histology.

1g. Harvest the liver, lung, kidney and brain tissues for determination of metastatic lesions by both IHC and PCR based techniques.

Task 1, Aim 2 Progress in First Year of Funding (1a – 1e), Year Two: Not yet initiated.

Proposed Work for Task 1, Year Two:
1. Begin animal husbandry.

Task 2 – Manuscript Preparation. Months 30-36
Task 2, Aim 2 Progress in First Year of Funding (1a – 1e), Year Two: Not yet initiated.

Proposed Work for Task 2, Year Two:
1. Begin outline of manuscript.
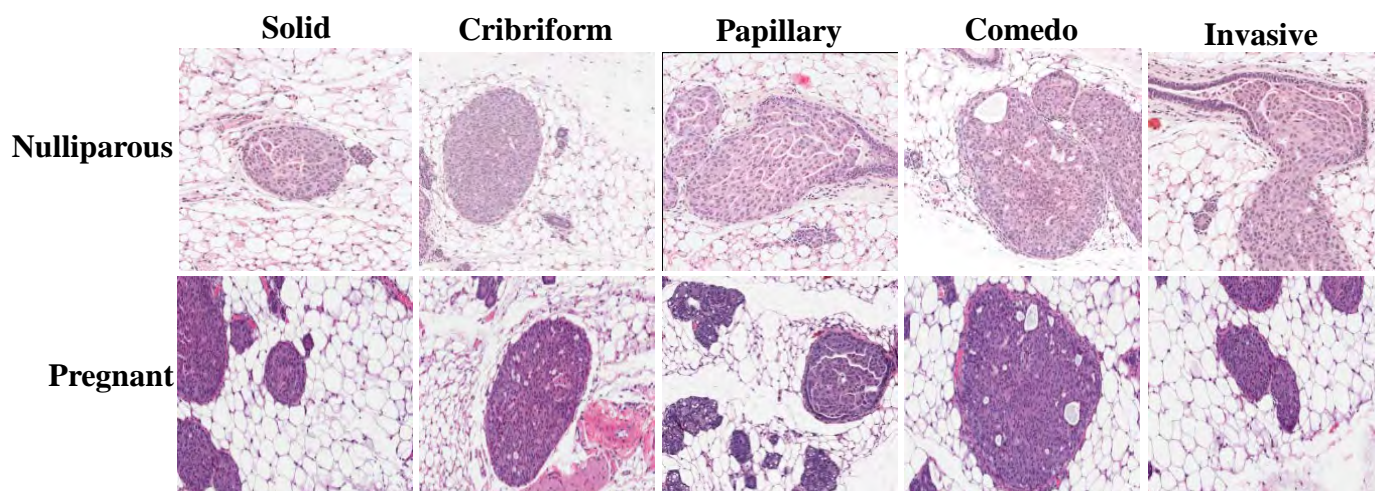
Figure 1. Hemotoxylin and eosin staining of mouse mammary glands intraductally injected with MCF10ADCIS.com tumor cells. MCFDCIS10A.com cells undergo stages of tumor development (solid, cribriform, papillary, comedo, and locally invasive) in nulliparous, (top panels) and pregnant (bottom panels) environments. Scale bar = 50 um.
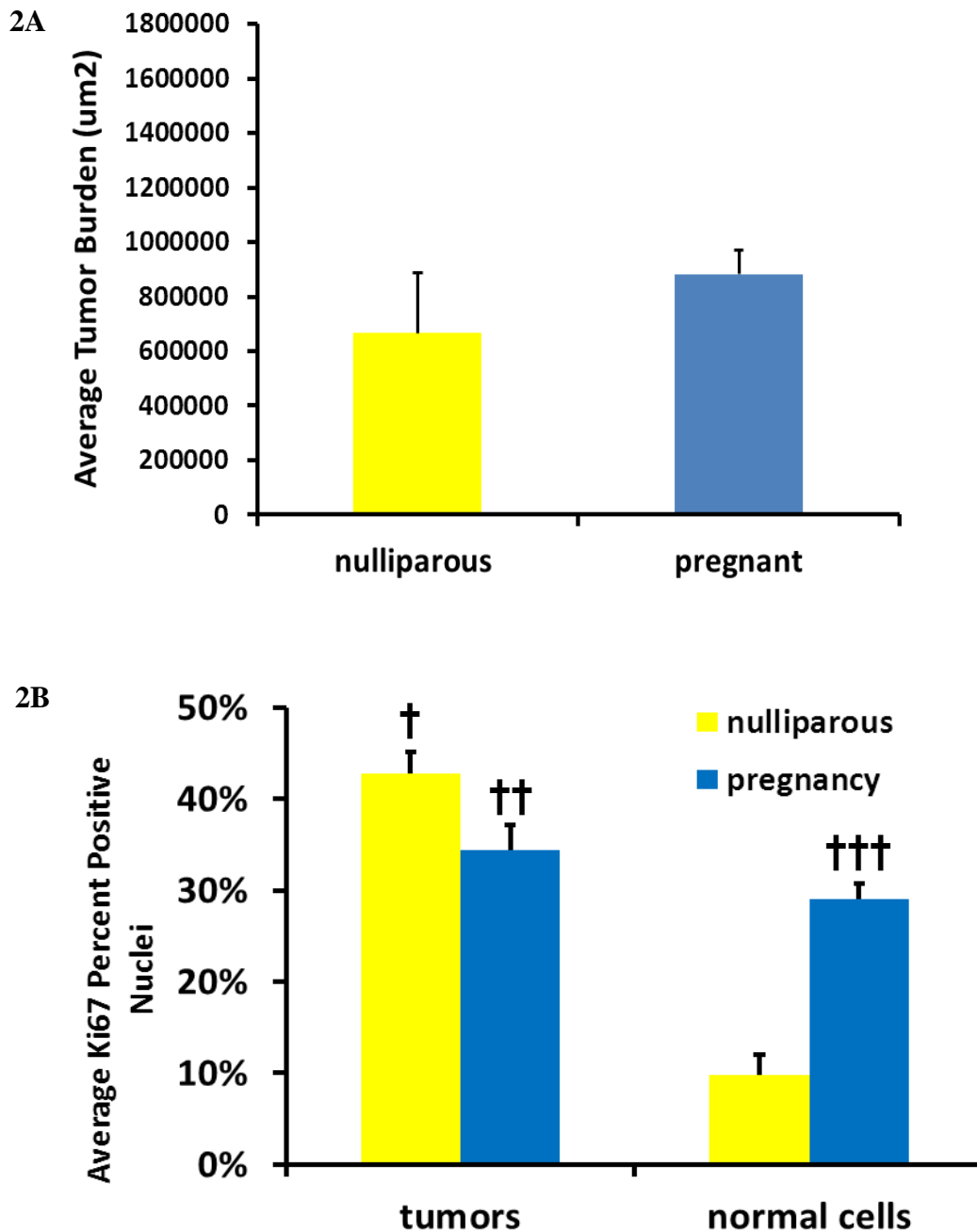
Figure 2. Effect of pregnancy on tumor burden and proliferation. (A) Tumor burden is not increased in the pregnant group compared to their respective nulliparous controls. (B) Ki67 proliferative index is increased in tumors from the nulliparous group compared to normal cells (†p = 1.956E-06). Interestingly, there was no difference in Ki67 proliferative index between tumors and normal cells in the pregnant group (p = 0.1256). There was a slight but significant decrease in Ki67 proliferative index in tumors from the pregnant group compared to tumors from their respective nulliparous controls (††p = 0.0441). As expected, Ki67 proliferative index was much higher in normal cells from the pregnant group compared to nulliparous controls (†††p = 1.407E-05). Error bars = SEM. Statistical analysis = Student's paired t-test.
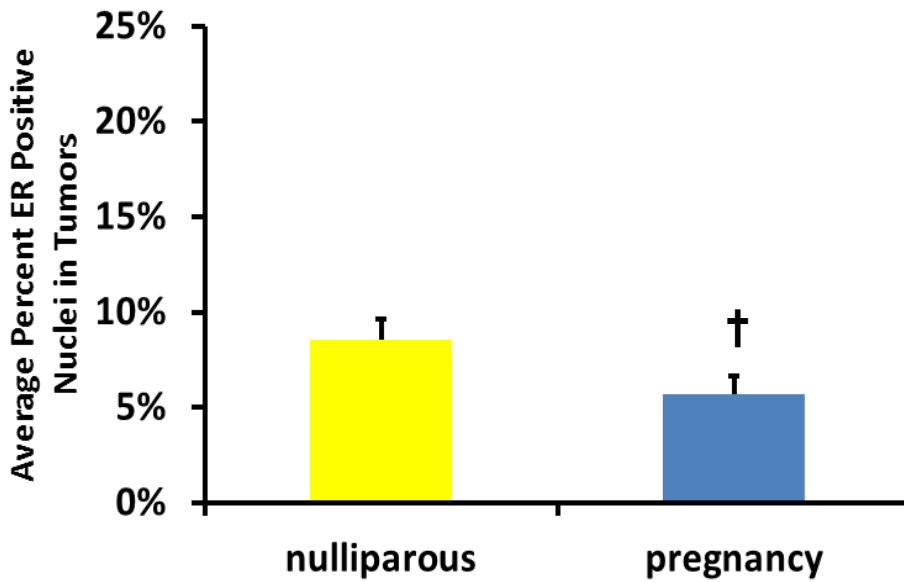
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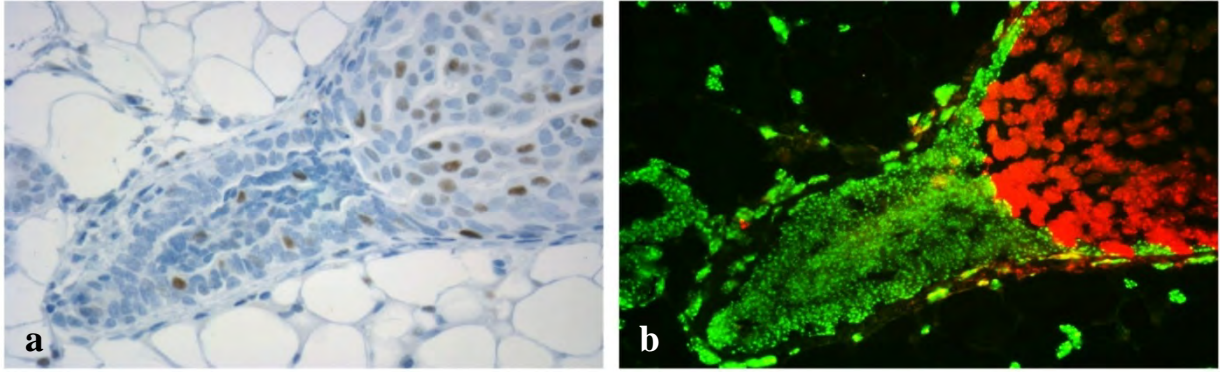
3B

Figure 3. Effect of pregnancy on ER re-expression. (A) IHC analysis of ER (a, brown) shows ER expressed in some, but not all, MCF10ADCIS.com tumor cells. These data were confirmed by FISH analysis (b) of human (red) and mouse (green) COT-1 DNA. (B) ER expression in MCF10ADCIS.com cells is slightly lower in the pregnant group compared to their respective nulliparous group (*p = 0.0697). Error bars = SEM. Statistical analysis = Student's paired t-test.
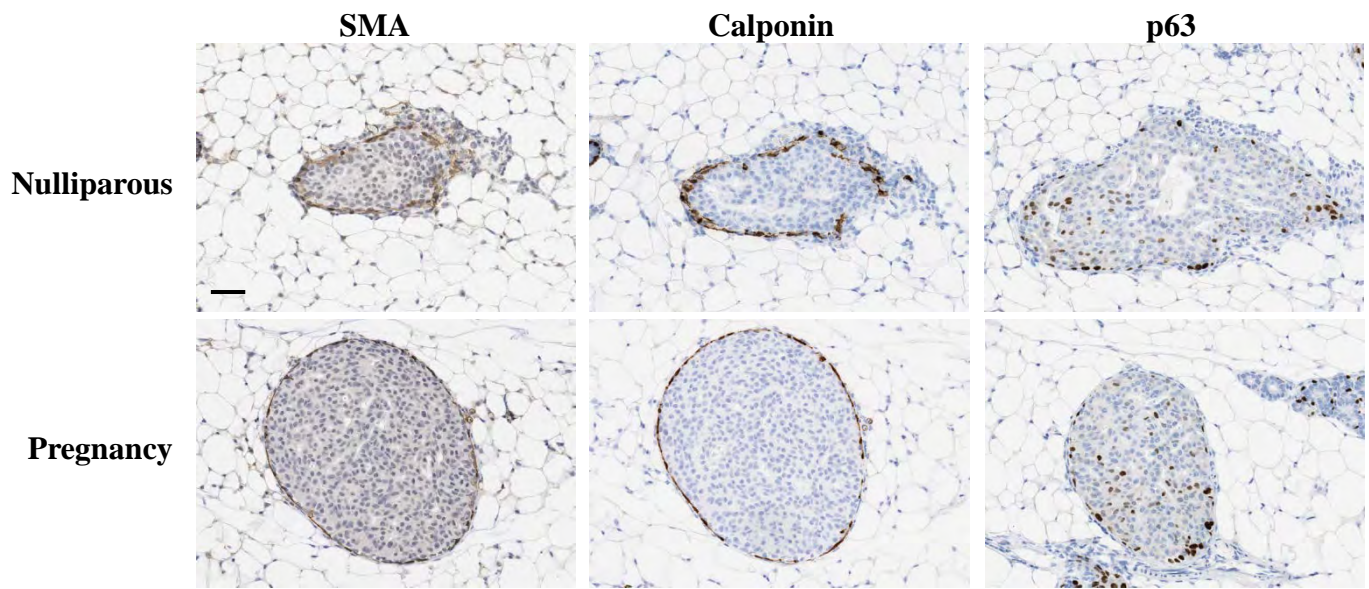
Figure 4. Myoepithelial cell layer markers outline normal ductal structures and tumors. Serial IHC analysis of myoepithelial cell markers smooth muscle actin (SMA; brown, left), calponin (brown, middle) and p63 (brown, right) outlining tumors in the nulliparous and pregnant host environments. Images scanned using Aperio software. Scale bars = 50 μm.

Table 1. Quantification of tumors surrounded by myopepithelial cell markers in nulliparous and pregnant host environments.

GROUP	>50% Tumors Surrounded by SMA	>50% Tumors Surrounded by Calponin	>50% Tumors Surrounded by p63
Nulliparous	60%	52%	40%
Pregnancy	92%	71%	46%

5A

| Solid | Cribriform | Papillary | Comedo | Invasive |

5B
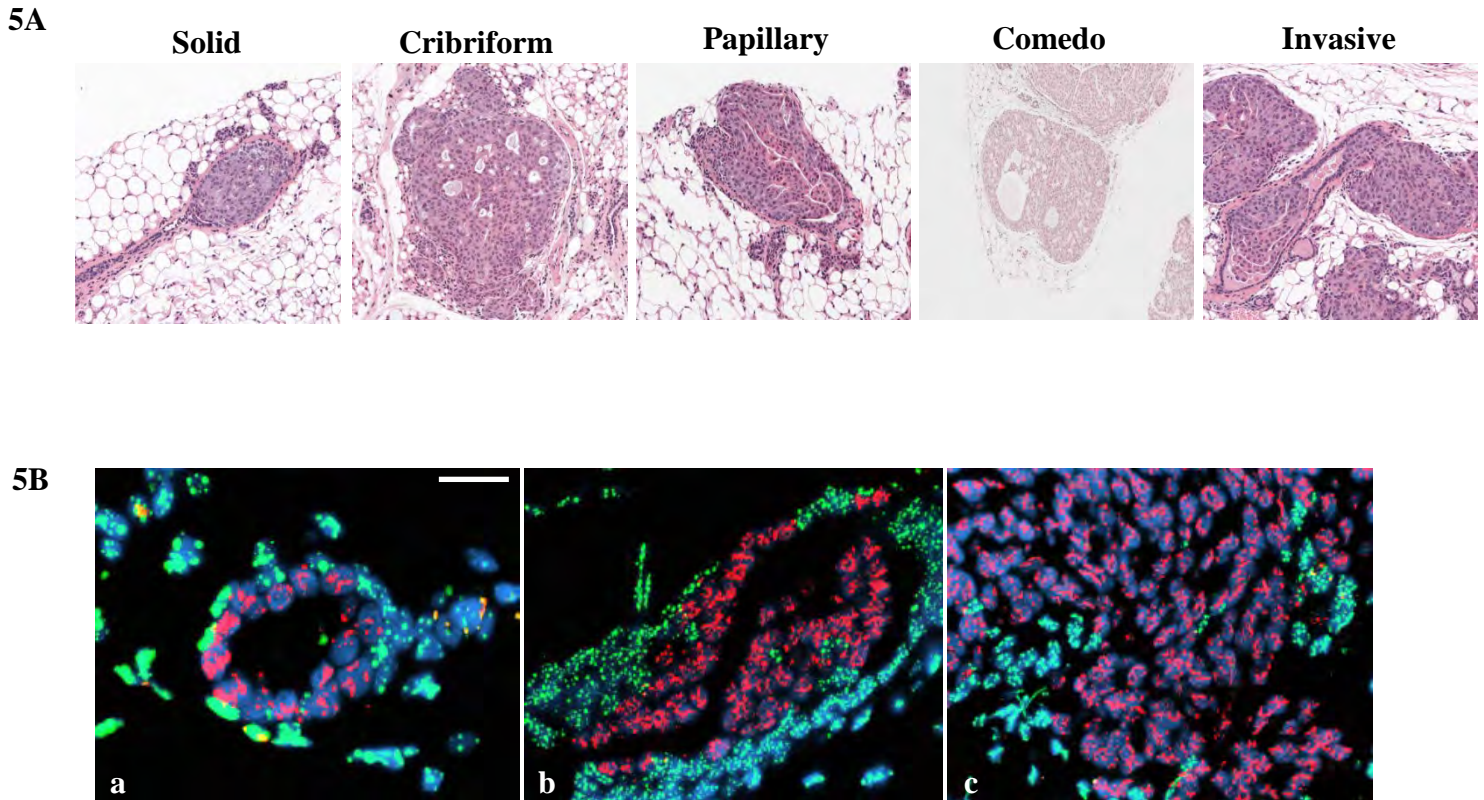
Figure 5. DCIS characteristics of MCF10ADCIS.com cells in the involution host environment. (A) MCFDCIS10A.com cells undergo stages of tumor development (solid, cribriform, papillary, comedo, and locally invasive) in the involution host environment. Similar DCIS characteristics are present in the nulliparous group (see Figure 1). (B) Fluorescent in situ Hybridization (FISH) detection of human and mouse cells in intraductal model. FISH analysis for COT-1 DNA (red = human COT-1; green = mouse COT-1) reveals evidence of tumor progression from (A) hyperplastic alveolar nodules (B) to ductal carcinoma situ to (C) local invasion. Scale bar = 40 µm.
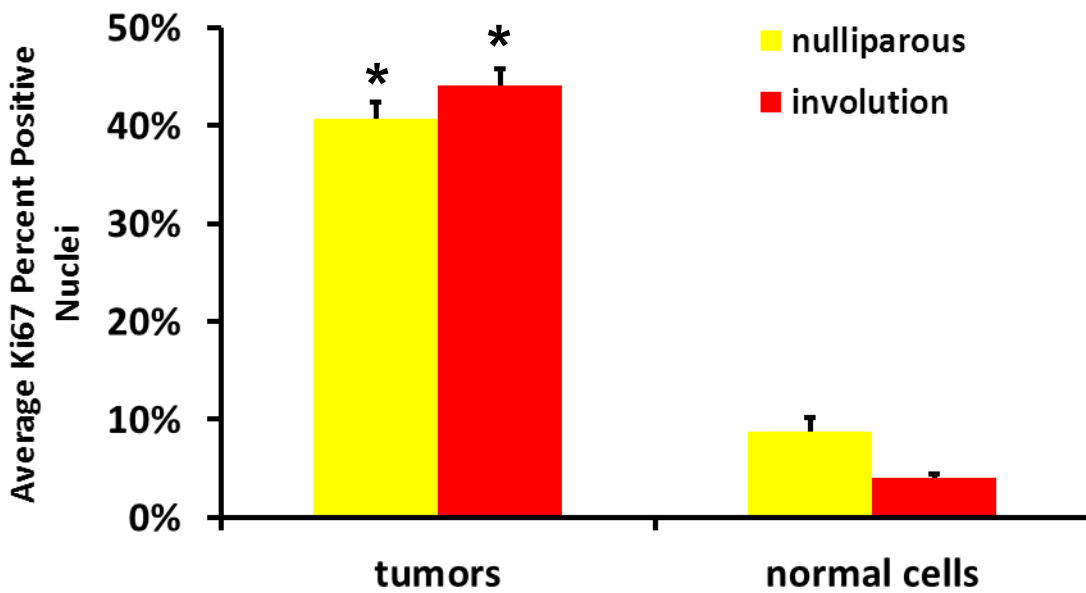
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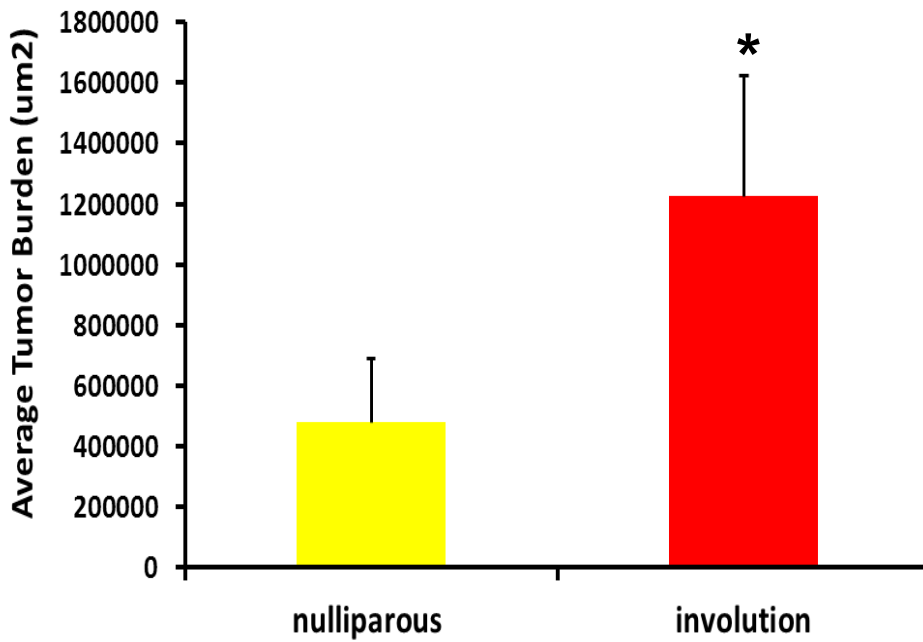
6B

Figure 6. Effect of involution on tumor burden and proliferation. (A) Tumor burden is greater in the involution group compared to their respective nulliparous controls (*p = .0637). (B) Ki67 proliferative index is increased in tumors from both the nulliparous and involution groups compared to normal cells (*p = 5.859E-08 and 1.082E-07, respectively). There was no significant difference in Ki67 proliferative index between tumors in the nulliparous group compared to the involution group (p = 0.1737). However, there was a decrease in Ki67 proliferative index in normal cells from the involution group compared to normal cells from their respective nulliparous controls (**p = 0.021). Error bars = SEM. Statistical analysis = Student's paired t-test.
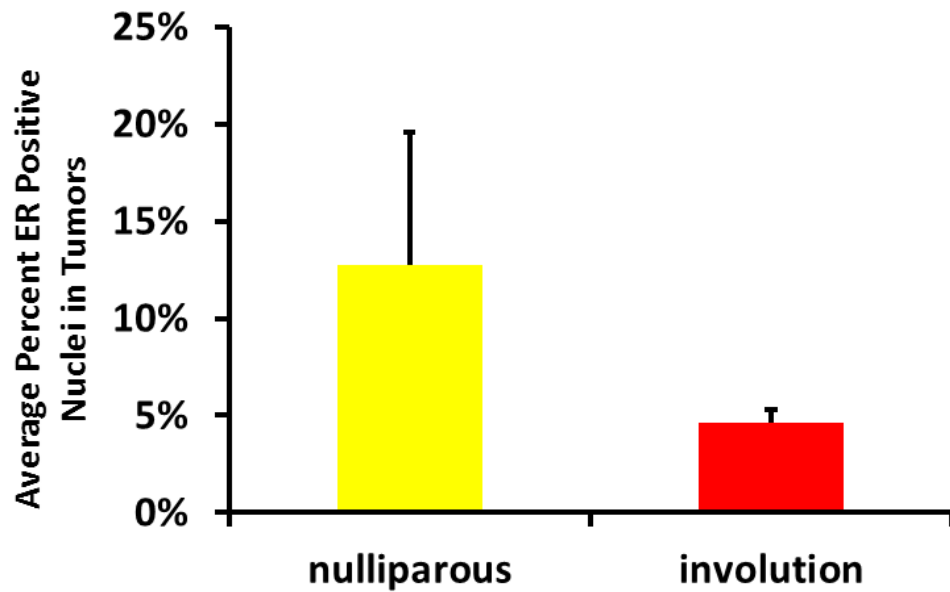
13

Figure 7. Effect of involution on ER re-expression. There was no difference in ER expression in tumors from the involution group compared to their respective nulliparous controls (*p = 0.3009). Error bars = SEM. Statistical analysis = Student's paired t-test.
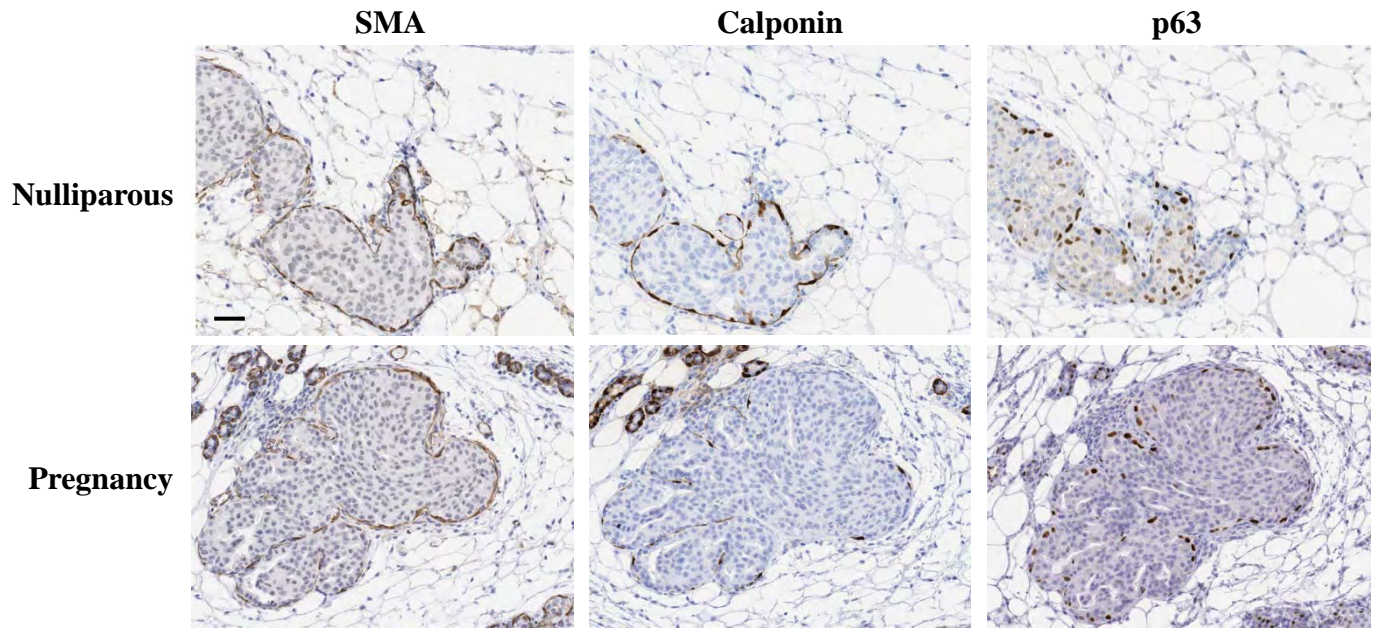
Figure 8. Myoepithelial cell layer markers outline normal ductal structures and tumors. Serial IHC analysis of myoepithelial cell markers smooth muscle actin (SMA; brown, left), calponin (brown, middle) and p63 (brown, right) outlining tumors in the nulliparous and involution host environments. Images scanned using Aperio software. Scale bars = 50 μm.

Table 2. Quantification of tumors surrounded by myopepithelial cell markers in nulliparous and pregnant host environments.

GROUP	>50% Tumors Surrounded by SMA	>50% Tumors Surrounded by Calponin	>50% Tumors Surrounded by p63
Nulliparous	88%	35%	24%
Involution	88%	40%	43%

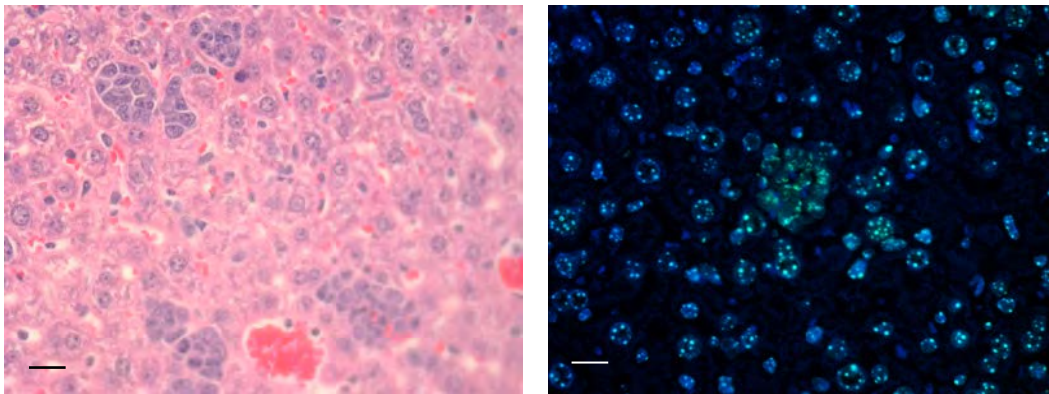| GROUP	% Tumors Surrounded by More p63 than Calponin	% Tumors Surrounded by Less p63 than Calponin	% Tumors Surrounded by the Same Amount of p63 than Calponin
Involution	53%	30%	15%

9A
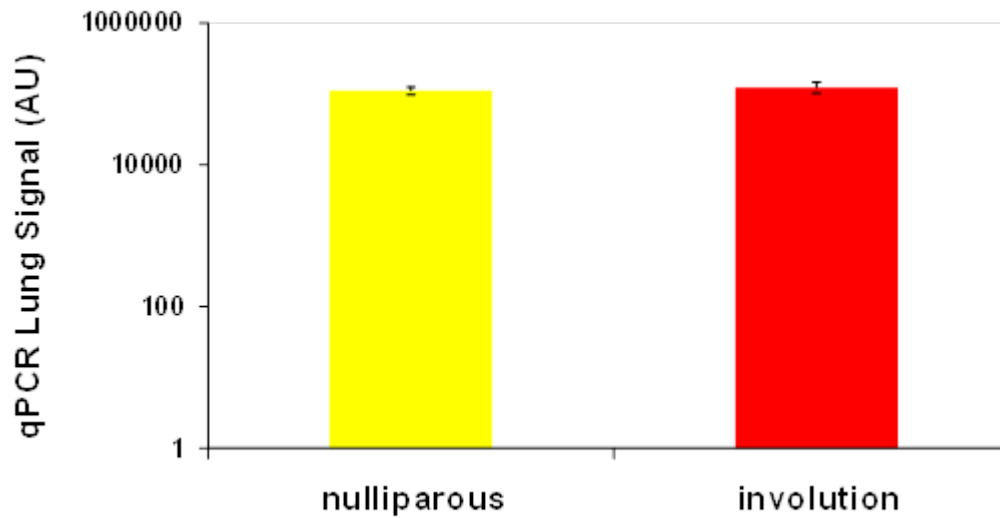
9B

Figure 9. No evidence of lung or liver metastasis in the nulliparous and involution host microenvironments. (A) qRT-PCR analysis of lung for human β2M transcripts in arbitrary units (a.u.) after normalizing to actin. No metastasis was detected in either the nulliparous (n=6) group or the involution (n=5) group (p = 0.642). Error bars = SEM. Statistical analysis = unpaired t test. (B) Hemotoxylin and eosin showed suspected areas of liver metastasis (panel a, arrows), but FISH analysis for COT-1 DNA (red = human COT-1; green = mouse COT-1) confirmed no metastatic lesions (panel b, arrows). Magnification = 40X. Scale bars = 20 um.

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KEY RESEARCH ACCOMPLISHMENTS

1. Successful development of a preclinical intraductal model of human breast cancer that minimizes wound healing programs or inflammation, which allows analysis of tumor cell progression within mammary ducts in the context of environmental changes associated with normal physiology.

2. Met the criteria for a model of human breast cancer progression, as MCF10ADCIS.com cells display histologic progression from hyperplastic alveolar nodules to invasive lesions in nulliparous, pregnant and involution hosts.

3. Evaluated tumor latency, growth, burden and multifocal nature of tumor during pregnancy and involution, showing differential effects of these reproductive states on these tumor characteristics.

4. Showed that tumor burden was not increased in the pregnant group compared to nulliparous controls, but is greater in mammary glands from the involution group compared to nulliparous controls. Tumors in the pregnant group appear to be less proliferative and have slightly lower ER expression than tumors in their respective nulliparous controls, suggesting that the hormonal milieu of pregnancy may have an effect on certain mechanisms of tumor progression.

5. Initiated characterization of the protective myoepithelial cell layer as a focal point of tumor progression. p63 positivity appears to be lost first in all tested microenvironments and calponin positivity appears to lost preferentially in the post-partum involution environment. These data may reflect key differences in the influence of these reproductive states on tumor progression.

REPORTABLE OUTCOMES

Manuscripts: In progress

Abstracts:
1. **Tanya D. Russell,** Jaime Fornetti, and Pepper Schedin**.** Does Pregnancy and Post-Partum Involution Promote Tumorigenesis in a Preclinical Model for Early Stage Human Breast Cancer? Department of Defense Era of Hope Conference, Orlando, Florida, August 2-5, 2011.

2. Pepper Schedin, Traci R. Lyons, Jenean O'Brien, **Tanya Russell**, Holly Martinson, Patricia J. Keely, and Virginia Borges. Postpartum mammary gland involution promotes breast tumor progression through collagen and COX-2 and is inhibited by NSAIDS. Department of Defense Era of Hope Conference, August 2-5, 2011.

3. V.F. Borges, T. Lyons, J. O'Brien, **T. Russell**, H. Martinson, P. Keely, P.J. Schedin. The Role of Collagen and COX-2 in post-partum breast involution on the progression of pregnancy-associated breast cancer and its inhibition by NSAIDs. ASCO Annual Meeting, June 3-7, 2011.

4. **Tanya D. Russell**, Sonali Jindal, Jaime Fornetti, and Pepper Schedin. A Preclinical Mammary Intraductal Model to Study Early Stage Human Breast Cancer. 102nd AACR Annual Meeting "Innovation and Collaboration: The Path to Progress", Orlando, Florida, April 2-6, 2011.

5. **Tanya D. Russell,** Sonali Jindal, Jaime Fornetti, and Pepper Schedin. Does Pregnancy and Post-Partum Involution Promote Tumorigenesis in a Preclinical Model for Early Stage Human Breast Cancer? University of Colorado Denver Mammary Gland Biology Retreat, Aurora, CO, January 20-21, 2011.

6. **Tanya D. Russell**, Jaime Fornetti, and Pepper Schedin. Pregnancy and Postpartum Involution Promote Tumorigenesis in a Preclinical Model for Triple Negative/Basal Breast Cancer. Third AACR Conference on "The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved", Miami, FL, September 30-October 3, 2010.

Oral Presentations:
1. **Tanya D. Russell.** Pregnancy and Involution Promote Tumorigenesis in a Preclinical Model for Early Stage Human Breast Cancer. January 2011 Research in Progress, Division of Medical Oncology, University of Colorado Denver.

2. **Tanya D. Russell.** Does Pregnancy and Post-Partum Involution Promote Tumorigenesis in a Preclinical Model for Early Stage Human Breast Cancer? January 2011 University of Colorado Denver Mammary Gland Biology Retreat, Aurora, Colorado.

3. **Tanya D. Russell.** Department of Health and Human Services (HHS) National Institutes of Health (NIH) National Cancer Institute (NCI) 2010 Cancer Research Imaging Camp June 20–25, 2010 St. Louis, MO.

Poster Presentations:
1. **Tanya D. Russell,** Jaime Fornetti, and Pepper Schedin. Does Pregnancy and Post-Partum Involution Promote Tumorigenesis in a Preclinical Model for Early Stage Human Breast Cancer? August 2011 Department of Defense Era of Hope Conference, Orlando World Marriot Center, Orlando, Florida.

2. **Tanya D. Russell,** Jaime Fornetti, and Pepper Schedin. Does Pregnancy and Post-Partum Involution Promote Tumorigenesis in a Preclinical Model for Early Stage Human Breast Cancer? June 2011 Gordon Research Conference on Mammary Gland Biology, Salve Regina University, Newport, Rhode Island.

3. **Tanya D. Russell**, Sonali Jindal, Jaime Fornetti, and Pepper Schedin. A Preclinical Mammary Intraductal Model to Study Early Stage Human Breast Cancer. April 2011 102nd AACR Annual Meeting "Innovation and Collaboration: The Path to Progress", Orlando, Florida.

4. **Tanya D. Russell**, Jaime Fornetti, and Pepper Schedin. Pregnancy and Postpartum Involution Promote Tumorigenesis in a Preclinical Model for Triple Negative/Basal Breast Cancer. 2010 UCD Third Annual Women's Health Research Day, Aurora, Colorado.

5. **Tanya D. Russell**, Jaime Fornetti, and Pepper Schedin. Pregnancy and Postpartum Involution Promote Tumorigenesis in a Preclinical Model for Triple Negative/Basal Breast Cancer. 2010 Third AACR Conference on the "Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved", Miami, Florida.

Awards: AACR Minority Scholar in Cancer Research Award - Third AACR Conference on the "Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved", 2010

Patents and licenses: none

Development of cell lines, tissue, serum repositories: none

Informatics: none

CONCLUSION

These preclinical data model the human epidemiological data on PABC. We have recently suggested that PABC be divided in two groups: cases diagnosed during pregnancy that have a better prognosis and cases diagnosed during the post-partum period which have a poorer prognosis [5]. Thus, these initial studies begin to help address the question of why breast cancer (especially in African American women), when diagnosed within close proximity to a recent pregnancy or in the absence of breastfeeding, is more aggressive and more likely to spread.

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APPENDICES: Abstracts

APPENDICES

ABSTRACTS:

Conference: DOD Era of Hope
Date: August 2-5, 2011
Location: Orlando, Florida

Does Pregnancy and Postpartum Involution Promote Tumorigenesis in a Preclinical Model for Early Stage Human Breast Cancer?

Tanya D. Russell, Sonali Jindal, Jaime Fornetti, and Pepper Schedin
Division of Medical Oncology, University of Colorado Denver Anschutz Medical Campus

Young African American women have an increased risk of developing and dying from "triple negative/basal breast cancer" compared to young Caucasian women, but the reason for this is unknown. For all women, having had a recent pregnancy is an independent risk factor for poor prognosis. Epidemiologic data shows increased risk of basal breast cancer with increased childbearing in African American women (Millikan et al 2008). We hypothesize that the hormones of pregnancy combined with the tissue-remodeling of the post-partum breast back to its pre-pregnant state provides a two-hit tumor promotional tissue environment that promotes triple negative/basal breast cancer.

To elucidate this hypothesis, we are developing a murine intraductal mammary model to examine the progression of early stage human breast cancer. Our intraductal model allows the study of tumor cell formation and dissemination from mammary ducts by delivering human mammary tumor cells directly through the intact mouse teat into the correct anatomical location for ductal in situ carcinoma (DCIS) without surgical manipulations. In contrast, other established intraductal models perform surgical manipulations to expose the teat canal which can be confounded by wound healing and inflammatory programs that are reported to be tumor promotional in other contexts.

Using the bi-potential progenitor, triple negative/basal MCF10ADCIS.com cell line and harvesting tissue 4 weeks post-injection, we corroborate reported studies showing DCIS lesions with distinct characteristics of the main human subtypes and progression to invasive disease. Immunohistochemical (IHC) analysis indicates that these cells incorporate into the mouse mammary ducts and form E-cadherin-based junctional complexes with neighboring cells, including other human tumor cells or normal mouse epithelial cells. Tumor burden in the postpartum involution group was much greater than the respective nulliparous control group. However, the Ki67 proliferative index is lower in the involution group compared to the respective nulliparous control group, suggesting a mechanism other than proliferation is driving tumor promotion in the postpartum setting. Tumor burden in the pregnancy group was not significantly different than nulliparous controls. Although these cells have been previously characterized as estrogen receptor (ER) negative both *in vitro* and *in vivo*, fluorescent in situ hybridization analysis revealed that some tumors developed within our model were ER positive according to the Allred scoring method.

In summary, our murine intraductal model of human breast cancer provides a rigorous approach to studying early stage tumor progression and is particularly suited to studying host effects on tumor progression. Since mammary ducts are the primary sites of occult tumors in women, we propose that this intraductal model will be a more relevant model for all stages of human breast cancer. These data also suggest that the physiological environment of involuting mammary glands promote tumor progression; further work is needed to elucidate the role of pregnancy on tumor progression. Our goal is to unfold the biological mechanisms responsible for the effects of parity on aggressive breast cancers and help reduce the breast cancer health disparity that exists in African American women. Future plans include the use of this innovative model to investigate the effects of lactation on triple negative/basal breast cancer.

Postpartum mammary gland involution promotes breast tumor progression through collagen and COX-2 and is inhibited by NSAIDS.

Pepper Schedin, Traci R. Lyons, Jenean O'Brien, **Tanya Russell**, Holly Martinson, Patricia J. Keely, and Virginia Borges.
Division of Medical Oncology, University of Colorado Denver Anschutz Medical Campus

The prevalence and mortality rate of "triple negative/basal breast cancer" is increased young African American women compared to young Caucasian women. We hypothesize that pregnancy and involution without lactation promotes triple negative/basal breast cancer. **Aims:** 1) Determine if pregnancy and involution, in the absence of lactation, confers tumor promotion. 2) Determine if lactation mitigates promotional effects of pregnancy/involution on tumor progression. **Results:** Compared to nulliparous controls, tumors in the pregnant group appear to be less proliferative and tumor burden is not increased. In contrast, tumors in the involution group were much more proliferative and tumor burden was increased with respect to nulliparous controls. The protective myoepithelial cell layer may be preferentially compromised by tumors formed in post-partum involution mammary glands. These data may reflect key differences in the influence of these reproductive states on tumor progression. Impact: Our initial studies help to address the question of why breast cancer (especially in African American women), when diagnosed within close proximity to a recent pregnancy, is more aggressive and more likely to spread.

A Preclinical Mammary Intraductal Model to Study Early Stage Human Breast Cancer

Tanya D. Russell, Sonali Jindal, Jaime Fornetti, and Pepper Schedin
Division of Medical Oncology, University of Colorado Denver Anschutz Medical Campus

Our goal is to develop a murine intraductal mammary model to examine the progression of early stage human breast cancer. Current models include injection of human mammary epithelial cell lines into the mammary fat pad (MFP) of immunocompromised mice or intraductal injection via cleaved nipple. With the MFP model, these cells are injected into the correct anatomical location for primary invasive tumors but do not form in the location of initiation, i.e., the mammary ducts. Intraductal models allow direct study of tumor cell formation and dissemination from mammary ducts; however, surgical manipulations performed to expose the teat canal are confounded by wound healing and inflammatory programs that are reported to be tumor promotional in other contexts.

Our intraductal model of human breast cancer delivers human mammary tumor cells directly through the mouse teat into the correct anatomical location for ductal in situ carcinoma (DCIS) without surgical manipulations. Further, our model provides co-evolution of tumor progression with stromal changes versus the MFP model where the stroma responds to tumor cells at time of injection. Using the bi-potential progenitor MCF10ADCIS.com cell line and harvesting tissue 4 weeks post-injection, we corroborate reported studies showing DCIS lesions with distinct characteristics of the main human subtypes and progression to invasive disease. Immunohistochemical (IHC) analysis shows that these cells incorporate into the mouse mammary ducts and form E-cadherin-based junctional complexes with neighboring cells, including other human tumor cells or normal mouse epithelial cells. Although these cells have been characterized as estrogen receptor (ER) negative both in vitro and in vivo, IHC revealed that some tumors developed within our model were ER positive according to the Allred scoring method.

Our model permits a rigorous evaluation of the effects that physiologic-induced changes in mammary tissue remodeling (i.e. postpartum involution) has on tumor progression. We investigated tumor incidence and burden in the postpartum involution setting and found much greater increases in the involution group compared to the respective nulliparous control group. Interestingly, the Ki67 proliferative index is lower in the involution compared to the respective nulliparous control, suggesting a mechanism other than proliferation is driving tumor promotion in the postpartum setting.

In summary, our murine intraductal model of human breast cancer provides a rigorous approach to studying early stage tumor progression. This model is particularly suited to studying host effects on tumor progression and future studies will determine the effects of physiological endocrine status on tumor cell dissemination from mammary ducts. Since mammary ducts are the primary sites of occult tumors in women, we propose that this teat injection model will be a more relevant model for all stages of human disease.

DOES PREGNANCY AND POSTPARTUM INVOLUTION PROMOTE TUMORIGENESIS IN A PRECLINICAL MODEL FOR EARLY STAGE HUMAN BREAST CANCER?

Tanya D. Russell, Sonali Jindal, Jaime Fornetti, and Pepper Schedin, Division of Medical Oncology University of Colorado Denver, Aurora, CO

Background: Young African American women have an increased risk of developing and dying from "triple negative/basal breast cancer" compared to young Caucasian women, but the reason for this is unknown. For all women, having had a recent pregnancy is an independent risk factor for poor prognosis. Epidemiologic data shows increased risk of basal breast cancer with increased childbearing in African American women (Millikan et al 2008).
Hypothesis: We speculate that the hormones of pregnancy combined with the tissue-remodeling of the post-partum breast back to its pre-pregnant state provides a two-hit tumor promotional tissue environment that promotes triple negative/basal breast cancer.
Results: Using an innovative, intraductal preclinical model developed in our laboratory, we tested whether a triple negative/basal breast cancer cell line (MCF10ADCIS.com) is promoted by pregnancy or postpartum involution. After harvesting tissue 4 weeks post-injection, we corroborate reported studies showing DCIS lesions with distinct characteristics of the main human subtypes and progression to invasive disease. Immunohistochemical (IHC) analysis indicates that these cells incorporate into the mouse mammary ducts and form E-cadherin-based junctional complexes with neighboring cells, including other human tumor cells or normal mouse epithelial cells. Tumor burden in the postpartum involution group was much greater than the respective nulliparous control group. However, the Ki67 proliferative index is lower in the involution group compared to the respective nulliparous control group, suggesting a mechanism other than proliferation is driving tumor promotion in the postpartum setting. Tumor burden in the pregnancy group was not significantly different than nulliparous controls. Although these cells have been previously characterized as estrogen receptor (ER) negative both *in vitro* and *in vivo*, fluorescent in situ hybridization analysis revealed that some tumors developed within our model were ER positive according to the Allred scoring method.
Conclusions/Future Directions: Our murine intraductal model of human breast cancer provides a rigorous approach to studying early stage tumor progression and is particularly suited to studying host effects on tumor progression. Since mammary ducts are the primary sites of occult tumors in women, we propose that this intraductal model will be a more relevant model for all stages of human breast cancer. These data also suggest that the physiological environment of involuting mammary glands promote tumor progression; further work is needed to eludicate the role of pregnancy on tumor progression. Our goal is to unfold the biological mechanisms responsible for the effects of parity on aggressive breast cancers and help reduce the breast cancer health disparity that exists in African American women. Future plans include the use of this innovative model to investigate the effects of lactation on triple negative/basal breast cancer.

Pregnancy and Postpartum Involution Promote Tumorigenesis in a Preclinical Model for Triple Negative/Basal Breast Cancer

Tanya D. Russell, Jaime Fornetti, and Pepper Schedin
Division of Medical Oncology, University of Colorado Denver, Aurora, CO

Young African American women have an increased risk of developing and dying from "triple negative/basal breast cancer" compared to young Caucasian women, but the reason for this is unknown. Insight into this problem may be elucidated by the fact that breast cancers associated with a recent pregnancy are more likely to be metastatic. For all women, having had a recent pregnancy is an independent risk factor for poor prognosis. Epidemiologic data shows increased risk of triple negative/basal breast cancer with increased childbearing in African American women (Millikan et al 2008). We speculate that the hormones of pregnancy combined with the tissue-remodeling of the post-partum breast back to its pre-pregnant state provides a two-hit tumor promotional tissue environment that results in increased metastasis of these tumors.

Using an innovative, intraductal preclinical model developed in our laboratory, we tested whether a triple negative/basal breast cancer cell line (MCF10ADCIS.com) is promoted by pregnancy or postpartum involution. When tumor cells are injected into mammary ducts of involuting female mice, there is evidence of tumor progression from hyperplastic alveolar nodules to ductal carcinoma in situ to local invasion that is accompanied by progressive loss of the protective myoepithelial cell layer characteristic of ductal carcinoma in situ. Tumor burden in the postpartum involution group was much greater than the respective nulliparous control group. Surprisingly, tumor burden in the pregnancy group was not significantly different than nulliparous controls. Nevertheless, with pregnancy and postpartum involution the protective myoepithelial cell layer surrounding tumors is compromised. Physiologic changes to adherens junctions and myoepithelial cells occur during normal pregnancy and post-partum involution, which may contribute to tumor progression observed with pregnancy and involution. Further, while involution is a normal tissue remodeling process that occurs after childbirth in the absence of breastfeeding, or at weaning in women who breastfeed, the process uses tissue remodeling and inflammatory programs associated with cancer progression. These data suggest that the physiological environments of pregnant and involuting glands promote tumor progression and begin to unfold the biological mechanisms for the effects of parity on aggressive breast cancers. Future plans include the use of this innovative model to investigate the effects of lactation on triple negative/basal breast cancer.